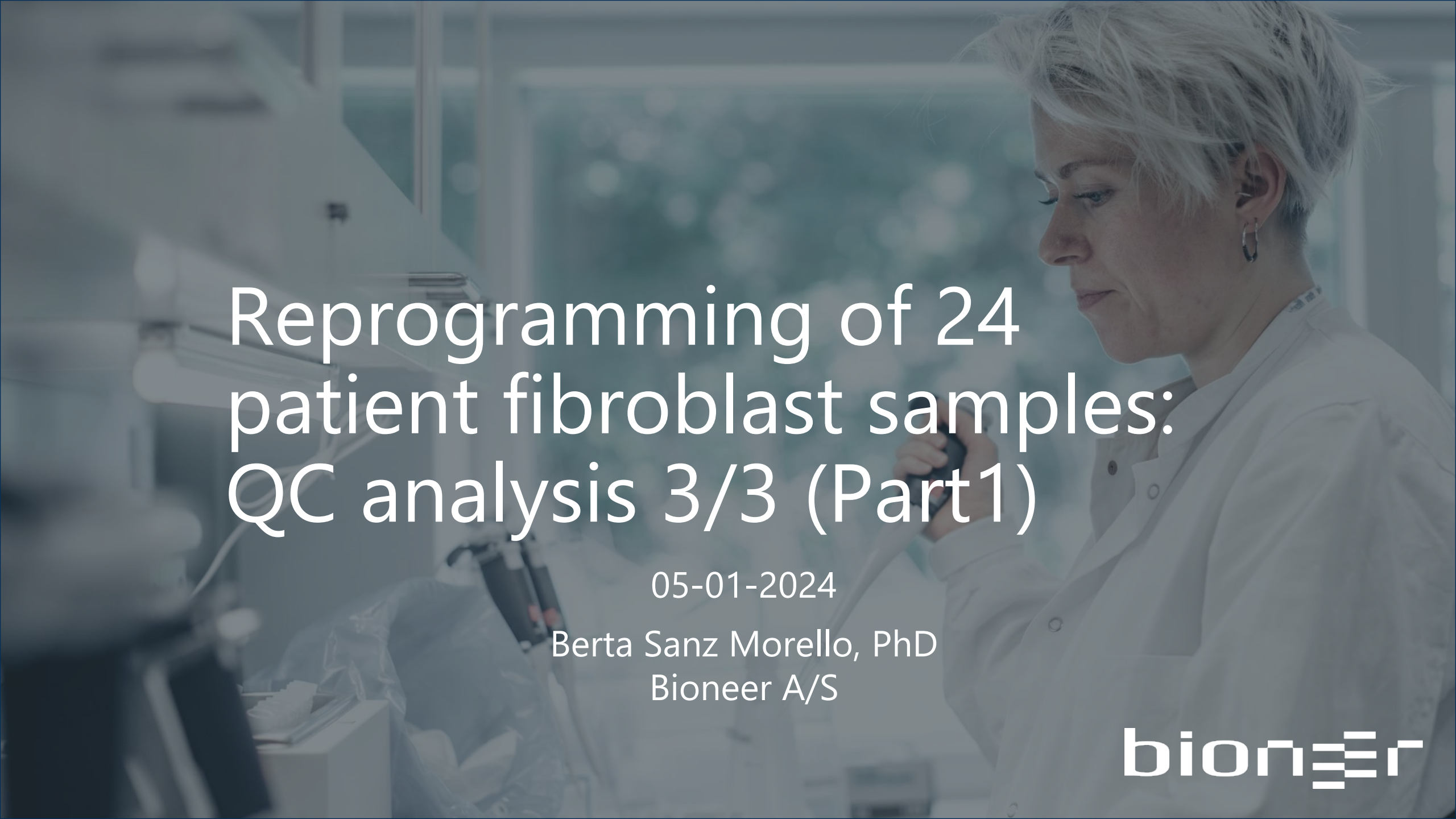


Reprogramming of 24 patient fibroblast samples: QC analysis 3/3 (Part1)

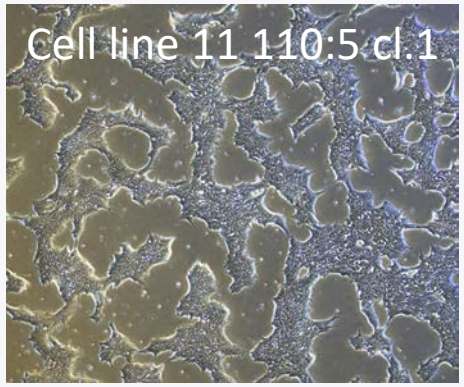
05-01-2024

Berta Sanz Morello, PhD
Bioneer A/S

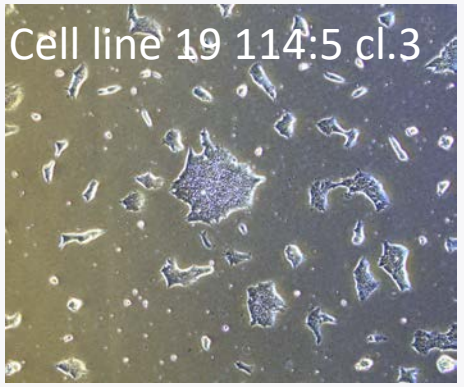
Morphology

Morphology of iPSCs 24 hours after thawing*:

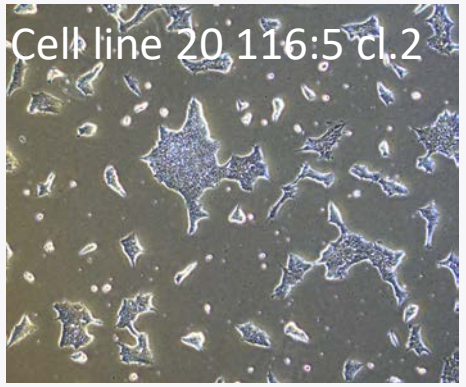
hPSCreg ID
SUHi013-A



hPSCreg ID
SUHi020-A



hPSCreg ID
SUHi021-A

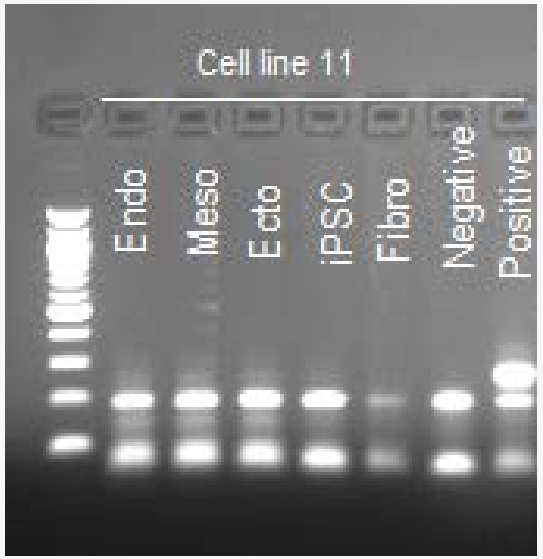


*Photos of the morphology of the iPSC lines before banking will be sent in a separate format (photos are too heavy to be included here).

QC Overview

hPSCreg ID	Line ID	Clone	Mycoplasma	Sterility	Trilineage diff.
SUHi013-A	Cell line 11 110:5	1	Passed	Passed	Passed
SUHi020-A	Cell line 19 114:5	3	Passed	Passed	Passed
SUHi021-A	Cell line 20 116:5	2	Passed	Passed	Passed

Mycoplasma test



hPSCreg ID	Line ID	Clone
SUHi013-A	Cell line 11 110:5	1
SUHi020-A	Cell line 19 114:5	3
SUHi021-A	Cell line 20 116:5	2

Sterility test

No contamination was detected in any of the lines 48 hours after thawing (data not shown).

Trilineage differentiation

		Fold over reference sample			
hPSCreg ID	SAMPLES	GATA4 (Endo and Meso marker)	CXCR4 (Meso and Endo marker)	Pax 6 (Ecto marker)	GAPDH
SUHi013-A	Cell line 11 110:5 cl 1 iPSC	1	1	1	1
SUHi020-A	Cell line 19 114:5 cl 3 iPSC	1	1	1	1
SUHi021-A	Cell line 20 116:5 cl 2 iPSC	1	1	1	1
SUHi013-A	Cell line 11 110:5 cl 1 Endo	1084,890209	46,3126156	7,981537412	1
SUHi020-A	Cell line 19 114:5 cl 3 Endo	4781,784284	87,02351186	3,758090997	1
SUHi021-A	Cell line 20 116:5 cl 2 Endo	1348,057825	89,47007579	42,91271673	1
SUHi013-A	Cell line 11 110:5 cl 1 Meso	283,3942481	217,7707932	0,065607293	1
SUHi020-A	Cell line 19 114:5 cl 3 Meso	1237,603386	449,8603275	0,069991975	1
SUHi021-A	Cell line 20 116:5 cl 2 Meso	901,8018546	473,318226	1,598442299	1
SUHi013-A	Cell line 11 110:5 cl 1 Ecto	0,117440344	39,48775199	4502,987216	1
SUHi020-A	Cell line 19 114:5 cl 3 Ecto	0,190782401	22,67975788	1584,706553	1
SUHi021-A	Cell line 20 116:5 cl 2 Ecto	0,108317131	19,38228379	10108,86451	1

Fold over reference sample is based on the formula:

$2^{-(\Delta Ct \text{ target tissue} - \Delta Ct \text{ reference tissue})}$

Where:
ΔCt = Ct gene of interest – Ct house keeping gene

Target tissue = endo, ecto or mesoderm
Reference tissue = undifferentiated iPSCs
Gene of interest = GATA4, CXCR4, or Pax6
Housekeeping gene = GAPDH

Trilineage differentiation

		Fold over reference sample			
hPSCreg ID	SAMPLES	GATA4 (Endo and Meso marker)	CXCR4 (Meso and Endo marker)	Pax 6 (Ecto marker)	GAPDH
SUHi013-A	Cell line 11 110:5 cl 1 iPSC	1	1	1	1
SUHi020-A	Cell line 19 114:5 cl 3 iPSC	1	1	1	1
SUHi021-A	Cell line 20 116:5 cl 2 iPSC	1	1	1	1
SUHi013-A	Cell line 11 110:5 cl 1 Endo	1084,890209	46,3126156	7,981537412	1
SUHi020-A	Cell line 19 114:5 cl 3 Endo	4781,784284	87,02351186	3,758090997	1
SUHi021-A	Cell line 20 116:5 cl 2 Endo	1348,057825	89,47007579	42,91271673	1
SUHi013-A	Cell line 11 110:5 cl 1 Meso	283,3942481	217,7707932	0,065607293	1
SUHi020-A	Cell line 19 114:5 cl 3 Meso	1237,603386	449,8603275	0,069991975	1
SUHi021-A	Cell line 20 116:5 cl 2 Meso	901,8018546	473,318226	1,598442299	1
SUHi013-A	Cell line 11 110:5 cl 1 Ecto	0,117440344	39,48775199	4502,987216	1
SUHi020-A	Cell line 19 114:5 cl 3 Ecto	0,190782401	22,67975788	1584,706553	1
SUHi021-A	Cell line 20 116:5 cl 2 Ecto	0,108317131	19,38228379	10108,86451	1

All iPSC lines tested were able to differentiate to the 3 germ layers.

Reprogramming of 24 patient fibroblast samples: QC analysis. Pluripotency test

22-03-2024

Berta Sanz Morello, PhD
Bioneer A/S

Pluripotency analysis

hPSCreg ID	SAMPLES	OCT4	NANOG	GAPDH
SUHi001-A	Cell line 1 143 cl 1	1.19	0.95	1
SUHi002-A	Cell line 2 145 cl 2	0.99	0.92	1
SUHi003-A	Cell line 3 146 cl 1	1.23	0.88	1
SUHi004-A	Cell line 4 152 cl 3	1.00	0.56	1
SUHi005-A	Cell line 5 165 cl 2	0.98	0.75	1
SUHi006-A	Cell line 6 169 cl 3	1.16	0.65	1
SUHi007-A	Cell line 7 102:5 cl 1	1.41	0.75	1
SUHi008-A	Cell line 8 104:5 cl 2	1.21	0.94	1
SUHi010-A	Cell line 9 117:5 cl 3	1.16	1.11	1
SUHi014-A	Cell line 10 120:5 cl 3	0.92	0.62	1
SUHi013-A	Cell line 11 110:5 cl 1	0.81	0.82	1
SUHi009-A	Cell line 12 105:5 cl 3	1.20	0.77	1
SUHi011-A	Cell line 13 151 cl 1	1.11	0.79	1
SUHi012-A	Cell line 14 163 cl 2	1.01	0.87	1
SUHi015-A	Cell line 15 141 cl 3	0.65	0.84	1
SUHi016-A	Cell line 16 142:3 cl 1	0.80	1.15	1
SUHi018-A	Cell line 17 RES168 cl 1	0.91	0.89	1
SUHi020-A	Cell line 19 RES114:5 cl 3	1.13	0.66	1
SUHi021-A	Cell line 20 RES116:5 cl 2	0.74	0.73	1
SUHi022-A	Cell line 21 RES118:5 cl 1	0.80	0.60	1
SUHi023-A	Cell line 22 RES121:5 cl 5	0.81	0.38	1
SUHi024-A	Cell line 23 RES174 cl 4	0.78	0.47	1
SUHi017-A	Cell line 24 RES158 cl 4	0.93	0.55	1
Control	Control iPSC line: BIONi010-C	1	1	1

Fold over reference sample is based on the formula:

$2^{-(\Delta Ct \text{ target tissue} - \Delta Ct \text{ reference tissue})}$

Where:
ΔCt = Ct gene of interest – Ct house keeping gene

Target tissue = reprogrammed cell lines
Reference tissue = iPSC line BIONi010-C
Genes of interest = OCT4, NANOG
Housekeeping gene = GAPDH

All reprogrammed lines tested showed pluripotency ability.